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# Paper-Strip Blood-Sampling Technique for the Detection of Antibody to the Plague Organism *Yersinia pestis*

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The paper-strip blood-sampling technique was evaluated for efficacy in plague passive hemagglutination tests. It is valuable for widespread serological surveys.

Filter paper techniques for the collection of whole bloods and sera for subsequent detection of antibodies are well documented. Techniques have been adapted for antibody studies of various viruses (1-3, 5, 6, 8), leptospires (7), and plague (4). Because of difficulties experienced in the preparation and elution of paper disks for plague studies, our laboratory investigated the efficacy of a simplified paper-strip technique developed by Kenzo Nobuto (Toyo Roshi Kaisha, Ltd., Tokyo, Japan).

Nobuto blood-sampling papers were used in accordance with the manufacturers recommendations. This paper consists of a 5- by 30-mm section for adsorption of 0.1 ml of whole blood or serum and a 10- by 18-mm section for support during collection and drying. The 5- by 30-mm section was saturated with blood or serum, shaken gently to remove excess fluid, and air-dried. Samples were eluted by cutting the 5- by 30-mm section in half and extracting overnight at 4 C; 0.4 ml of borate buffer at pH 8.0 was used for blood samples, and 0.2 ml of normal physiological saline was used for serum samples. Vials containing the diluent and paper strip were inactivated at 56 C for 30 min. The paper strip was pressed to the bottom of the vial with a glass rod, and the extract was adsorbed with washed sheep erythrocytes (1:10, vol/vol) for 20 min at room temperature. After centrifugation the supernatant fluids were tested for the presence of antibody to the water-soluble fraction 1A envelope protein of *Yersinia pestis*. Passive hemagglutination tests and hemagglutination inhibition controls were those recommended in the protocol described by the World Health Organization (9). Results are shown in Tables 1-3.

There is good agreement between parallel serum and serum-strip antibody titers, but there is a tendency to overestimate titers obtained by the paper-strip collection method. Parallel tests of 24 samples on day 1 yielded mean titers of 1:446 and 1:592 for sera and serum strips, respectively (Table 1). Titers were very stable, showing only moderate loss of antibody during the 1-year test period; mean titers for the 24 positive sera changed from 1:592 to 1:244. Storage of the nine normal sera on paper strips for the 1 year did not result in the appearance of nonspecific agglutinins.

Comparison of titers for whole blood strips and their respective sera yielded consistent results within the limits expected for the passive hemagglutination method (Table 2).

The passive hemagglutination test has been

TABLE 1. *Geometric means and range of serum titers obtained from whole sera and filter paper-strip samples stored at ambient temperatures and humidities*

| Serum | | Storage period | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 Day | | | | 6 Months | | 1 Year | |
| Type | No. | Serum titer | | Paper-strip titer | | Paper-strip titer | | Paper-strip titer | |
| | | GMT | Range | GMT | Range | GMT | Range | GMT | Range |
| Normal | 10 | NEG[b] | | NEG | | NEG | | NEG | |
| Antiplague | 24 | 1:446 | 1:16-1:8192 | 1:592 | 1:16-1:16384 | 1:322 | 1:16-1:8192 | 1:244 | 1:16-1:4096 |

[a] GMT, Geometric mean titer.
[b] NEG, <1:8.

TABLE 2. *Results of tests for comparison of serum titers with titers obtained from whole bloods on filter paper strips*

| Passive hemagglutination titers (sera) | Passive hemagglutination titers (filter paper strips [whole blood]) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | <1:32 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 | 1:1024 | 1:2048 | 1:4096 |
| <1:8 | 15[a] | | | | | | | | |
| 1:8 | 1 1[b] | | | | | | | | |
| 1:16 | 1 | 2 | | | | | | | |
| 1:32 | | 2 | | | | | | | |
| 1:64 | | | | 1 2 | | | | | |
| 1:128 | | | | 1 | 1 | | | | |
| 1:256 | | | | 1 | 2 2 | | | | |
| 1:512 | | | | | 1 | 1 | | | |
| 1:1024 | | | | | | 1 | 1 | | |
| 1:2048 | | | | | | | 2 | | |
| 1:4096 | | | | | | | | | 2 |

[a] Numbers which are not underlined, *Peromyscus* sp. surviving laboratory challenge with *Y. pestis* strain CS 445.
[b] Number underlined, vaccinated human subjects.

used to measure infection rates in small mammal populations and to assess the geographic range of plague infection in small rodents and carnivores. Certain technical restrictions make it inadvisable to use paper-strip techniques to measure infection rates. Unavoidable dilution occurs during extraction of the paper strips which results in dilutions of 1:4 and 1:8 for sera and whole bloods, respectively. The application of passive hemagglutination microtechniques according to World Health Organization protocols results in an additional fourfold dilution; minimal test titers are 1:16 for sera and 1:32 for whole bloods. Tests for specificity, in which passive hemagglutination inhibition controls are used, require a minimum of three twofold dilutions. Thus, the minimal provable titers are 1:64 and 1:128—titers well above generally accepted diagnostic levels of 1:10 and 1:16. For surveillance purposes, however, if given sufficient numbers of positives in the test population, the portion of the population falling below the levels of 1:64 or 1:128 may be sacrificed without seriously affecting the overall results.

The practicality of this approach has been checked in work to be published in detail elsewhere. Very briefly, using the filter paper-strip technique, 778 carnivore and 210 small rodent blood specimens were collected by coworkers in the U.S. Fish and Wildlife Service,

TABLE 3. *Results of Y. pestis passive hemagglutination tests performed on whole blood specimens collected on Nobuto filter paper strips*

| Species | Results (titers) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Negative | Questionable[a] | | Positive[b] | | | | |
| | <1:32 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 | 1:1024 | ≥1:2048 |
| *Canis latrans* | 609 | 8 | 14 | 7 | 2 | 3 | 2 | 4 |
| *Lynx rufus* | 32 | 1 | 2 | | | | 1 | |
| *Mephitis* sp. | 27 | | 2 | 1 | | | | |
| *Procyon lotor* | 27 | | | 1 | | | | 4 |
| *Taxidea taxis* | 10 | | | | | | | |
| *Vulpes* sp. | 17 | | 2 | | 2 | | | |
| *Rattus rattus*[c] | 43 | 1 | 2 | 3 | 2 | | | |
| *Microtus californicus* | 153 | | 5 | 1 | | | | |
| Totals | 918 | 10 | 27 | 13 | 6 | 3 | 3 | 8 |

[a] Not verifiable by use of passive hemagglutination inhibition test.
[b] Verifiable by use of passive hemagglutination inhibition test.
[c] Sera collected and shipped on paper strips.

as well as in various other state and government agencies. Table 3 gives details of the serological results obtained by processing these specimens. In general, 37 potentially positive specimens were found at titers less than 1:128, as contrasted with 33 positive specimens with titers of 1:128 or greater. It appears that slightly more than 50% of the expected positive sera may be lost when the paper-strip technique is used.

In spite of this, filter-paper collection of whole blood samples has several advantages which counterbalance its failure to detect animals with low titers. For widespread serological surveys, methods of collection and handling are simple and personnel training is minimal. Amounts of blood required (0.1 ml) are small. Road-killed or poisoned animals, as well as animals collected in dead-fall traps, can be used. Access to laboratory facilities and refrigeration is not required; therefore, dried specimens can be mailed in simple envelopes without concern for delays in transit.

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